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PROCEEDINGS
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*PARTIAL SELF-INCOMPATIBILITY IN MEDICAGO SATIVA**

By R. A. Brink and D. C. Cooper

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Communicated October 3, 1938

It has been known since the work of Piper, Evans, McKee and Morse[1] that cross-pollination of alfalfa increases the production of seed, as compared with close-pollination. Heretofore, the cause of this behavior has not been revealed. In the present paper it will be shown that the difference is associated with two phenomena, possibly unrelated, whose effects can be distinguished by a histological study of the pistil following the two types of matings. The plant is found to be partially self-incompatible (self-sterile); that is, male gametophytes, while not impotent on the individual from which they arise, are less effective in accomplishing fertilization than are unrelated male gametophytes. The second important reason for the difference in fertility, as measured by the number of functional seeds developed, is the markedly greater tendency for ovules containing inbred embryos rather than those resulting from cross-pollination to collapse during development.

The seven plants used in the experiment were the immediate offspring, by close-pollination, of six unrelated individuals selected from commercial varieties of alfalfa. They were grown in a greenhouse at 70–80°F., and the pollinations were made in April, 1938, at the height of the flowering period. Newly opened flowers on several inflorescences on each individual were castrated. Some of these were then close-pollinated by using pollen from other flowers on the same respective plants. The remaining castrated flowers were cross-pollinated, the pollen being derived in each case from a different individual within the group. Each of the plants, therefore, served as a pistillate and as a staminate parent in the crosses, in addition to being selfed. In making the matings, an abundance of pollen was brought into intimate contact with the stigmatic secretion by means of toothpicks tipped with a narrow strip of fine emery cloth. The pistils were collected at 30, 48, 72, 96, 120 and 144 hours after pollination, fixed and embedded in paraffin. After sectioning and staining, the follow-

ing data were taken on each pistil, giving regard to the serial order of the ovules from apex to base of the ovary: position of the most advanced pollen tubes, fertility of the ovules, fertile ovules collapsing and stage of development of the embryo. About nine pistils per plant were taken at each collection in the selfed series and about six pistils in the crossed group.

Few pollen tubes advance beyond the mid-region of the ovary after selfing, whereas, following cross-pollination, the tubes usually reach the base of the ovarian cavity. At the time of the first collection, 30 hours after pollination, the longest pollen tubes in the selfed series had reached nearly to the fourth ovule, on the average. At 48 hours the mean position lay between the fifth and sixth ovules. Pollen tubes were not found in the later collections, growth being completed, presumably, prior to 72 hours in both series. Following cross-pollination, on the other hand, the leading pollen tubes were found between the eighth and ninth ovules at 30 hours, and at the tenth ovule 18 hours later. In both series the frequency of ovules becoming fertile declines from apex to base of the ovary, particularly beyond the fourth or fifth ovule.

Counts made on ovaries taken at 72 hours, at which time pollen-tube growth has ceased, and at 96, 120 and 144 hours, show that the eggs in 66 per cent of the ovules were fertilized in the cross-pollinated series, as compared with only 14.5 per cent after selfing. All seven plants, while varying in degree of self-incompatibility, reacted alike in showing a large increase in percentage of fertile ovules on being cross-pollinated. The disparity is related in part, to pollen-tube length. That this factor, however, does not fully account for the difference is shown below.

Pollen tubes usually were found to have reached at least the first four ovules at the apical end of the ovary following both self-pollination and cross-pollination. Tabulation of the condition of the ovules in these positions at the intervals beyond 48 hours reveals the fact, however, that only 28 per cent became fertile after selfing, in contrast with 80 per cent after crossing. In numerous cases in the selfed series pollen tubes were seen which had passed directly by the micropyle of an ovule containing an unfertilized egg. This tendency of a pollen tube which has reached the micropyle of a normal ovule not to enter is a manifestation of self-incompatibility which seems not to have been established definitely for any other plant. Stout and Chandler[2] have shown that incompatibility reactions very probably occur in the ovary following certain matings in *Hemerocallis*. Little appears to be known in these cases, however, concerning the behavior of the pollen tubes after the ovarian cavity is entered.

Not all the alfalfa ovules in which development of an embryo and endosperm is initiated at the time of fertilization continue growth. It is of

interest that the mortality is far higher among those containing inbred embryos. In the collections made after 48 hours, 34 per cent of the fertile ovules in the selfed series were found to be collapsing, as compared with only 7 per cent following cross-pollination. Whether the higher mortality of fertile ovules after selfing is correlated with the self-incompatibility or is an inbreeding depression effect cannot be stated definitely from the present evidence. The results of a previous study[3] of 10 plants in which no relationship was apparent between the proportion of ovules, becoming fertile after close-pollination and the frequency of abortion up to 120 hours suggest, however, that cessation of growth of the ovules may be an independent phenomenon.

Seed-formation in the alfalfa plant varies to an extraordinary degree, and the problem has yielded rather slowly to the attempts which have been made to elucidate it. It now appears that much of the difficulty which investigators have experienced in arranging well-directed experiments, and in interpreting the evidence from them, is due to the fact that more than one phase of the reproductive process is commonly subject to disturbance. Armstrong and White[4] have called attention to the stigmatic membrane as a factor conditioning pollen-germination, and some additional observations by the present authors[3] give further emphasis to this structure as an important variable. It is now shown that the alfalfa plant is partially self-incompatible. This fact also has far-reaching implications. A third, possibly independent, element which is significant for fertility is the collapse of fertile ovules during development. An effective approach to the general problem of seed-setting requires that attention be given to these distinct factors.

* Papers from the Department of Genetics, Agricultural Experiment Station, No. 234, and the Department of Botany, University of Wisconsin. The authors desire to express their appreciation for the support received from the Wisconsin Alumni Research Fund.

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MITOTIC BEHAVIOR OF INDUCED CHROMOSOMAL FRAGMENTS LACKING SPINDLE ATTACHMENTS IN THE NEUROBLASTS OF THE GRASSHOPPER

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Communicated October 14, 1938

The subject of the mitotic behavior and ultimate fate of x-ray induced fragments[1] has been almost entirely overlooked, or at least neglected, by most cytologists. Usually it is dismissed with the statement that fragments will not survive mitosis. This may be due in part to the use of material unsuitable for such studies, in part to the fact that fragments behave differently in different cells and in part to the interest of the investigator in other aspects of fragmentation. At most it has been noted that fragments lag at anaphase and are often included in small accessory nuclei at telophase.

The present study is based entirely on observations of the neuroblasts of the grasshopper, *Chortophaga viridifasciata*. Embryos were irradiated with 250 r, removed from the egg at such a time that cells treated in interphase or early prophase were in their first division, smeared on glass slips, fixed and stained in aceto-carmine, and mounted in euparal.

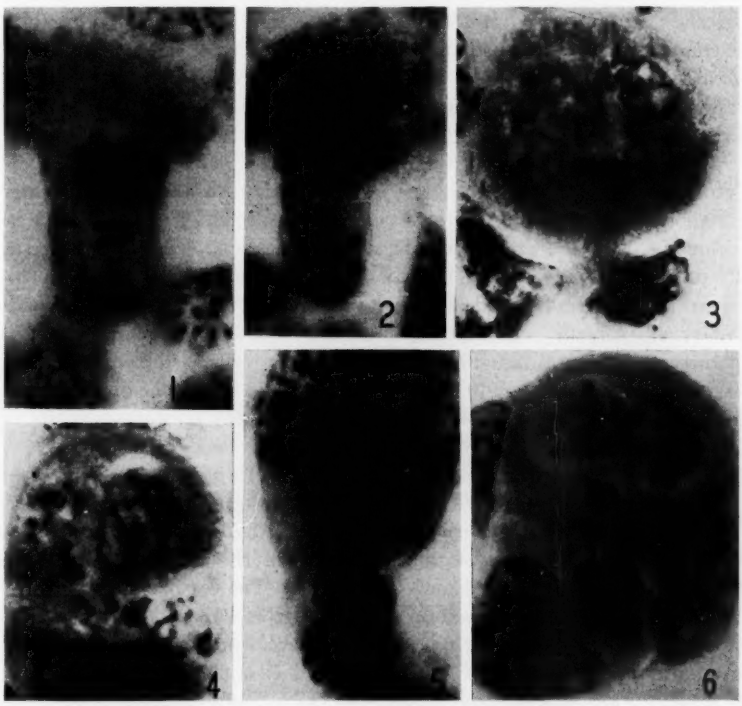
The results of this study throw light on certain factors involved in the mechanism of mitosis, because we are dealing here with chromosomal elements in which one complicating factor, namely, the spindle attachment region, is absent. Also, they indicate that the occurrence of delayed effects resulting from reattachments between chromosomes and fragments in a later mitotic cycle than that in which breakage occurred is not untenable cytologically.

Observations.—1. *Prophase.*—Fragments first become identifiable in the late prophase, when they differ from the chromosomes in that they lack the proximal heteropyknotic regions that mark the positions of spindle attachments. There is nothing in their behavior, their position in the nucleus, or their degree of condensation at this period to distinguish them from the chromosomes.

2. *Metaphase.*—The fragments lie between the cell membrane and the distal ends of the chromosomes. They are situated in the equatorial plane or at least as close to it as the distal ends of certain of the longest chromosomes, which often project slightly toward one or the other of the poles.

3. *Anaphase.*—A detailed analysis of the behavior of fragments at early anaphase appears in another paper.[2] The initial separation of their

"chromatids" begins at the same time as that of the chromosomes and gives rise to fragments of three classes: V's, rings and pairs of rods (Fig. 1). At first these are situated, as at metaphase, outside the distal ends of the



FIGURES 1–6

Fragments during and at the end of the first mitosis following their production. Dosage 250 r × 990. 1—Anaphase. Two pairs of daughter fragments. Those in focus have not yet become oriented in relation to the spindle. Of the pair out of focus at left the lower member is oriented parallel to the spindle axis. 2—Anaphase. Three pairs of daughter fragments on the spindle moving toward the poles. The upper member of the large pair at left is much nearer the pole than any of the others. 3—Telophase. The darkly staining fragments are included in the cell nucleus. 4—Telophase. One or more fragments lying partly outside the cell nucleus appear joined to chromosomes inside it. 5—Telophase. Accessory nuclei in neuroblast and ganglion cell, probably containing sister fragments. 6—Interphase. Neuroblast with accessory nucleus containing dark staining fragments.

chromosomes and therefore a considerable distance from the spindle. At late anaphase, however, after the daughter chromosomes have become well

separated with an accompanying elongation of the cell, the fragments move inward between their distal ends, eventually coming in contact with the spindle. Once this occurs their "chromatids" gradually become oriented in a line with the spindle axis and begin to move toward opposite poles (Fig. 2). If the "chromatids" open out as a pair of rods, separation is complete from the first, and the daughter fragments can move unhindered toward the poles. If the fragment is V- or ring-shaped, with fusion at one or both ends of the fragment, respectively, this union may persist for a time, so that the fragment lags at the equator for a varying period.

4. *Telophase.*—The position of the cleavage plane that divides the cytosome of the original neuroblast into daughter neuroblast and daughter ganglion cell determines in which cell a given daughter fragment will be included. Unless a great many fragments with complex fusion patterns are present, sister fragments usually come to lie in different cells (Fig. 5). In one embryo studied 17 cells in late anaphase and early telophase contained 81 pairs of "chromatid" fragments. Sister "chromatids" of 51 of these pairs were situated in different daughter cells, sister "chromatids" of 8 lay in the same daughter cell, while the apportionment of the "chromatids" of 22 pairs could not be determined with any reasonable degree of certainty. Coincident with cytokinesis is the loss of stainability of chromosomes and fragments and the gradual formation of clear areas about them, the extent of which mark the limits of the future nucleus. This occurs in the fragments somewhat later than in the chromosomes. Fragments that are close to the distal ends of the chromosomes are included with them in the cell nucleus (Fig. 3). The conclusion of Mather[3] in his studies of the post-meiotic resting stage of *Tradescantia, Eremurus* and *Allium*, therefore, that the absence of accessory nuclei is proof that irradiation took place after the last division does not apply to my material. Fragments that lie somewhat apart from the chromosomes are enclosed in small accessory nuclei (Fig. 5). In one embryo containing 52 neuroblasts in middle and late telophase the fragments are contained in 59 accessory nuclei and in 10 of the cell nuclei. Thirty-seven per cent of the former and 40% of the latter exhibit a stainability comparable to that of normal chromosomes, while the remaining 63 and 60%, respectively, are pyknotic, resembling in this respect the more deeply staining chromosomes of the ganglion cell nucleus (Fig. 6). There are also intermediate types in which one end of the fragment lies out in a small accessory nucleus, while the other end lies within the cell nucleus and appears to have become joined to the distal end of a chromosome (Fig. 4). Of the 59 accessory nuclei and 10 cell nuclei referred to above, 30% of the former and 60% of the latter show connections between fragments and chromosomes. This suggests delayed attachment.

5. *Interphase.*—Fragments persist throughout the interphase as dark staining elements contained within either accessory nuclei (Fig. 6) or cell nuclei, depending on their final location at late telophase.

Fragments and the Mechanism of Mitosis.—Any hypotheses bearing on the mechanism of mitosis must not overlook certain parallels that are manifest in the behavior of chromosomes, which possess spindle attachments, and fragments, which lack them. The facts must not be disregarded that (1) the fragments lie in the equatorial plane at metaphase, (2) their "chromatids" begin to separate at anaphase at the same time as do those of the chromosomes, (3) their "chromatids" come into intimate contact with the spindle at middle anaphase, and (4) sister "chromatid" fragments usually move toward opposite poles behind the chromosomes and so are included at telophase in different daughter cells.

(1) Many cytologists who have studied the mechanism of mitosis have been inclined to view the movement of the chromosomes into the equatorial plane at the end of the prophase as a force of some kind exerted by the spindle—in conjunction with the poles—on the chromosome through its spindle attachment. The regularity with which fragments, which lack spindle attachments and have no contact with the spindle, come to lie in the equatorial plane at metaphase, while it does not disprove the existence of an influence exerted by the spindle through the spindle attachment of the chromosome, nevertheless does demonstrate that other factors may be involved. To account for the orientation of the chromosomes in the equatorial plane at metaphase, Lillie[4] developed the hypothesis that the poles and chromosomes are electronegative, while the mid-region of the spindle is electropositive, the metaphase orientation resulting from the equilibrium established between these repelling and attracting forces. Darlington[5] holds that "the arrangement on the metaphase plate must be due to repulsion from the poles acting on the centromeres." Repelling forces, whatever their nature, between poles and chromosomes and between chromosomes *inter se* seem to offer the most reasonable explanation of the metaphase location of fragments. Forces must not be limited, however, to an action through the spindle and spindle attachments, since the fragments lie outside the spindle and lack spindle attachments. Their location in the peripheral part of the metaphase plate outside the other chromosomes suggests polar and interchromosomal repulsions, while their failure to lie within the spindle is doubtless due to the absence of spindle attachments. During cytokinesis vortical currents of the protoplasm are known to pass from the poles to the equatorial plane near the cell periphery, inward at the equator and poleward along the spindle. Currents at metaphase in the outer region of the cell moving toward the equator might carry fragments to the equatorial region, where they would

be held in the slower moving or stationary protoplasm among the distal ends of the chromosomes and between them and the cell membrane. It is a question, however, to what extent currents are present at metaphase. Bělař[6] stated that in the grasshopper spermatocyte they usually begin at early anaphase, though sometimes earlier or later than this. I have no evidence regarding this in the grasshopper neuroblast.

(2) Bělař,[6,7] Bleier,[8] Schaede[9] and Schrader[10] have come to the conclusion, from observations of a variety of material, that at least the initial separation of chromosomes is autonomous. The separation of the fragments, which occurs simultaneously with that of the chromosomes, demonstrates conclusively that, at least in these neuroblasts, the initial separation of "chromatids" can occur even though a spindle attachment region and a connection with the spindle are absent. This indicates, then, unless one assumes that other outer forces in the cell are the effective factors, that the forces causing chromatid separation reside within the chromosome itself, and so the act is autonomous. This evidence is not in accord with the views of Mather and Stone,[11] Darlington,[5] Upcott[12] and others, who hold that the anaphase separation of chromatids is invariably determined by the division and mutual repulsion of the spindle attachment bodies at the end of the metaphase.

(3) Fragments appear to be pushed against the spindle as a result of the decrease in equatorial diameter of the cell that accompanies its axial elongation at anaphase. This movement may be aided by protoplasmic currents passing inward at the equator. Just what connection is finally established between fragment and spindle is difficult to determine. While daughter chromosomes at anaphase have their distal ends rounded and their proximal ends pointed, as if they were continuous with a spindle fibre, both ends of daughter fragments frequently show an encircling fringe of dark-staining material, resembling, though perhaps only superficially, the ends of the anaphase meiotic chromosomes described by Schrader in *Protortonia* and by Hughes-Schrader in *Llaveia*.[13] The "Stemmkoerper" hypothesis of Bělař[6,7] seems to offer the most satisfactory explanation of such structures in fragments. When daughter fragments that have separated get among the outer fibres of a "Stemmkoerper" that is actively elongating and therefore tends to exert axial forces and lateral pressure against the sides of these elements, the edges at their ends might be pushed outward as encircling fringes. It seems improbable that any more of a connection between fragment and spindle exists than a close contact, which is effective in altering the position or shape of the fragment only in so far as there is pressure and friction between the two.

(4) Once the fragments have come in contact with the spindle, their tendency to become oriented parallel to its axis, their final separation

and their subsequent movement away from each other in the direction of the poles, may possibly be due, at least in part, to currents passing poleward. The hypothesis proposed by Schaede[9] could account for this behavior, since, according to it, poleward moving streams of protoplasm within the spindle are assumed to carry the daughter chromosomes to the poles. Vortical currents passing from the equator along the outer sides of the spindle to the poles might have a part in the poleward movements of daughter fragments not lying entirely within the spindle. Bělař,[6] who investigated the relation of these to chromosome movement, came to the conclusion, however, that the anaphase movements of the chromosomes are entirely independent of such currents. He attributed the middle and late anaphase movements of the daughter chromosomes to the elongation of the "Stemmkoerper." The poleward movement of fragments, if it is not the result of currents, supports Bělař's hypothesis at the same time that it is at variance with hypotheses positing only a pulling action of the spindle fibres; for it seems less likely that these elements, which apparently have no true attachment to the spindle, should be pulled toward the poles by contracting spindle components than that they should be pushed along because of their contacts with an elongating "Stemmkoerper." Bleier[8] attributed the whole anaphase movement of the daughter chromosomes to repelling forces of some kind originating in the chromosomes. It is true that this could account for the anaphase movements of fragments, though it fails to explain why the daughter fragments become oriented parallel to the spindle axis and delay their poleward movement until they come in contact with the spindle. The same difficulty confronts hypotheses of anaphase movement based on the presence of attracting forces between the poles and the chromosomes.

The main difference in the behavior of chromosomes with attachments and fragments without them is that the former move toward the poles more rapidly, and their points of attachment to the spindle lie at all times in a plane at right angles to the spindle axis, while the daughter fragments move toward the poles more slowly and with less regularity, and never arrive at a point as near the poles as the normal chromosomes. The conclusion seems justifiable, therefore, that in these cells, at least, the functions of the kinetochores are primarily to make uniform the orientation of the chromosomes in the equatorial plane at metaphase and to synchronize the middle and late anaphase separation of daughter chromosomes in order to insure their equal apportionment to the daughter cells, and not to effect their initial anaphase separation.

Delayed Effects.—In another paper[2] I have demonstrated that broken ends of chromosomes possessing spindle attachments can be transmitted, through the formation and breaking of chromatin bridges, to the second

cell generation following their production. It has been shown in the present paper that daughter fragments may be included at telophase in the cell nucleus with the chromosomes. McClintock[14] has demonstrated in *Zea* that broken ends of chromosomes retain their tendency to fuse and undergo unions from generation to generation. If the same is true of fragments, all the chromosomal conditions necessary for the occurrence of delayed attachment, as postulated by Stadler,[15] are fulfilled. In the present paper cells have been described that exhibit what are probably delayed attachments between fragments and chromosomes (Fig. 4).

I have no direct evidence regarding the behavior or fate of fragments beyond the mitotic cycle in which they appear. If, failing to become attached to a chromosome during that interphase, they fail to survive the next division, delayed effects of this kind will be limited to the chromosomes of the second cell generation after irradiation. If they may persist, undergo division and be included in the cell nucleus at the end of the second division after their formation, there is no reason why they may not survive through further cell generations, with the possibility at any time of delayed attachment with chromosomes at their broken ends. Some cells show sister fragments passing into different daughter cells seven days after irradiation, and it seems likely that these are undergoing their second division after irradiation.

Helwig[16] found a sufficient number of different chromosomal alterations represented in the secondary spermatogonia of single cysts, all the cells of which are descended from a single irradiated primary spermatogonium, to conclude that "fragmentation of the chromatin does not necessarily occur at the time of irradiation, but may be delayed." Up to the present time there has been no positive demonstration of delayed fragmentation except in some relatively rare cases of the breaking of a chromatin bridge in two places, so that a fragment lags at the cell equator. In this study and another,[2] however, I have found, in the persistence from one cell generation to the next of chromosomes and fragments with broken ends, a cytological basis for the occurrence of delayed attachments. The suggestion seems justifiable, therefore, that the delayed effects observed by Helwig might be interpreted in this way.

Summary.—The mitotic behavior of x-ray induced fragments lacking spindle attachments parallels that of the unaltered chromosomes of the grasshopper neuroblast in several respects. Sister "chromatids" of fragments separate at anaphase and are usually included in different daughter cells at telophase. Not infrequently they are included in the newly formed cell nucleus. This behavior has a bearing on certain hypotheses of the mechanism of mitosis and on the question of delayed reattachments following fragmentation.

[1] Throughout this paper the term *fragment* is used to designate a portion of a chromosome resulting from one or more breaks in an original chromosome and having no spindle attachment. The term *chromosome* is applied to an original chromosome or the part of an original chromosome containing a spindle attachment.

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A TITANOTHERE FROM THE TYPE SESPE OF CALIFORNIA

BY CHESTER STOCK

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Communicated October 7, 1938

Introduction.—In previous papers,[1] published mostly in these PROCEEDINGS, have appeared some of the paleontological results of explorations conducted in the Sespe deposits of Southern California. While the field efforts south of the Santa Clara Valley, Ventura County, were rewarded by rather startling results, no small amount of irritation was felt because of failure to find fossil vertebrate remains in the Sespe at the type locality north of the Santa Clara Valley. For, as is now known, the Sespe is not of same age throughout its stratigraphic thickness or at the several localities where fossil mammals have been found in it. It is, in fact, a series of beds that range in age from at least the upper Eocene to apparently the lower Miocene. Thus it seems especially important to determine by means of vertebrate evidence the age relationships of the type Sespe on Sespe Creek to that portion of the Sespe whose age is already established south of the Santa Clara Valley.

With this problem in mind the rugged terrain north of the Ojai Valley,

Ventura County, and in the immediate vicinity of Sespe Gorge was critically examined during the summer of 1937 by a field party of the California Institute of Technology. The outcome of this work was the discovery of titanothere remains in the lower portion of the Sespe, including a palate with teeth permitting a comparison with the titanotheres known from lower Tertiary horizons of southern California.

Geologic Occurrence.—The titanothere remains were encountered in massive gray-brown to pink sandstones of the Sespe on Sespe Creek (Plate 1). These heavily bedded and well indurated strata are conformable to the sandstones and shales of the marine upper Eocene. The uppermost marine formation in contact with the Sespe has been recognized as the Coldwater on the basis of invertebrate fossils and stratigraphic position by F. E. Dreyer,[2] who, prior to the discovery of the vertebrate remains, had studied the geology of the region. Deformation and structural complications in the immediate vicinity of the fossil locality make it difficult to determine the exact position of the titanothere remains in the Sespe above the contact with the Coldwater. However, it can be stated with some assurance that the location is not less than 400 feet nor more than 700 feet above the base of the Sespe.

DESCRIPTION OF MATERIAL

Teleodus cf. californicus Stock

Specimen.—A portion of a skull representing principally the palate with upper dentition, No. 2143 C. I. T. Vert. Pale. Coll., Plate 2, figs. A and B.

Locality.—C. I. T. Vert. Pale. Loc. 292, near north boundary of NE$^1/_4$, Sec. 2, R. 23 W., T. 5 N., San Bernardino B. & M., Mt. Pinos Quadrangle, Calif.

Comparisons.—The specimen, No. 2143, represents a mature individual in which the first molar and anterior premolars show considerable wear. The incisors and canines are likewise worn.

Two incisors are present on either side of the median line and these teeth possess the rounded, non-cingulate crowns seen in *Teleodus californicus*.[3] The lateral incisor is larger than the medial one. The canines possess relatively small crowns. This tooth resembles the canine in *T. californicus*, but the crown is actually of smaller size. A slightly longer diastema is present in No. 2143 than in No. 1834 C. I. T. Vert. Pale. Coll., from the Sespe uppermost Eocene beds (C. I. T. Vert. Pale. Loc. 150) north of the Simi Valley. Unfortunately the premolars are considerably worn. $P\underline{1}$ is not present on the right side and but poorly preserved on the left. What remains of the tooth suggests a triangular shaped crown and a size like that in *T. californicus*. It is possible that $P\underline{1}$ was slightly larger in No. 2143 than in individuals from locality 150. Resemblance between the

latter species and the present specimen is seen likewise in the size and shape of the posterior premolars.

Comparison of the molars in No. 2143 with those of *T. californicus* from the Sespe north of the Simi Valley reveals again a close similarity between these two forms in size, shape and in details of structure of the tooth crowns. As in the latter species the hypocone is distinct in $M\underline{3}$.

Comparing No. 2143 with a skull of *Teleodus uintensis*, No. 8634 Car-

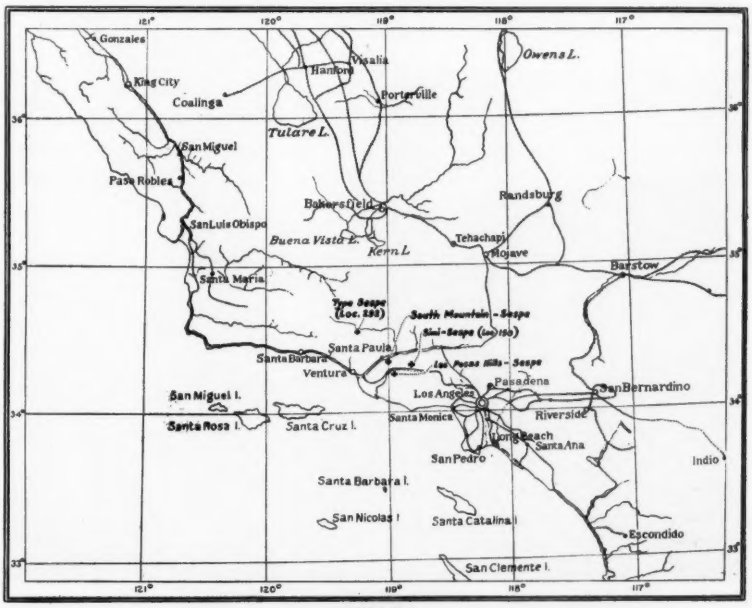

FIGURE 1

Index map showing location of principal vertebrate fossil occurrences in Sespe deposits of California. Note particularly position of localities 292 and 150.

negie Museum, from the Duchesne River formation of Utah, it is seen that certain differences in proportion exist between the two. Thus, while there is resemblance in width of snout across the canines and to some extent also in the length of the tooth-row, C–$M\underline{3}$ inclusive, the distance between the border of the maxillo-nasal notch and the anterior border of the orbit is shorter and the orbit is smaller in the California specimen.

No. 2143 is distinctly smaller than *Protitanops curryi* from the Titus Canyon formation, California. In the presence of larger incisors and in the shape of the anterior premolars the Sespe form is less advanced than the latter.

Conclusions.—A comparison of the titanothere remains from locality 292 with those previously described from the Californian area suggests a close similarity in stage of evolution to *Teleodus californicus* from the Sespe north of the Simi Valley. The differences between these two forms may be established ultimately as of specific value but their importance on the basis of available material does not appear to be greater than that resulting from variation within a single specific group.

In the light of this similarity one may conclude that that portion of the Sespe in which locality 292 occurs north of the Santa Clara Valley is

PLATE 1

View looking northeast across Sespe Creek showing typical exposure of heavily bedded sandstones of the Sespe continental beds resting on the Coldwater marine Eocene, and locality where titanothere was found.

closely related in time to the stage of the Sespe containing the fauna found at locality 150 south of the Santa Clara Valley. The assemblage of fossil mammals found at the latter locality has been regarded as of uppermost Eocene age. Its relationships are with the Duchesne River fauna of Utah and its position in the faunal sequence of western North America is between the Uinta (Uinta C) upper Eocene and the White River lower Oligocene.

Underlying the Sespe in the region of locality 292 is the marine Coldwater and the latter has come to be regarded as upper Eocene in age. In fact, W. P. Woodring[4] considers the Coldwater of the western Santa Ynez

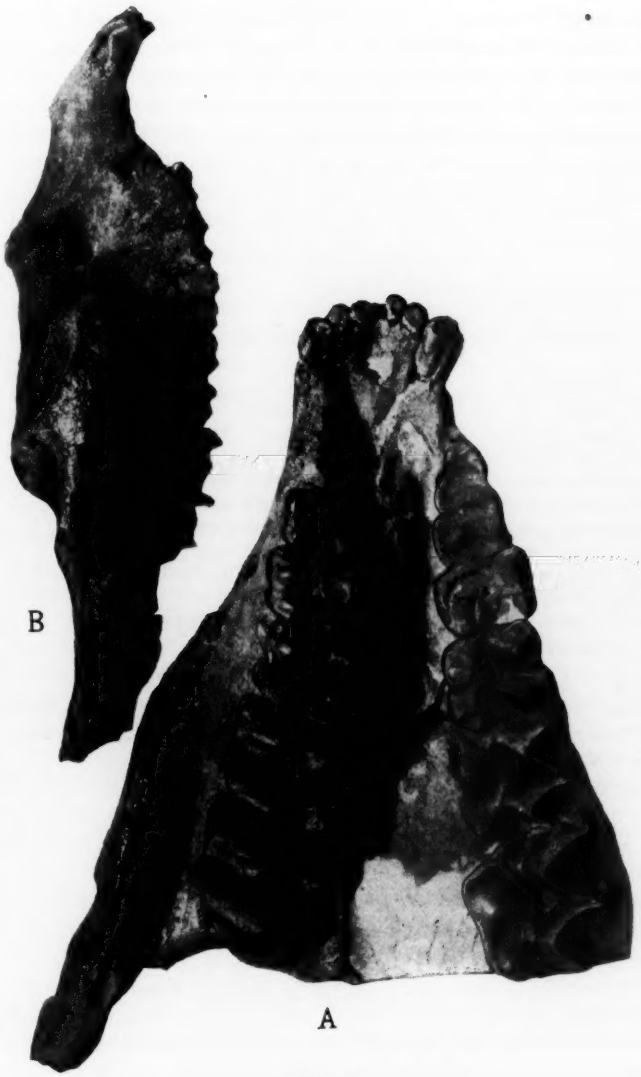

PLATE 2

Teleodus cf. californicus Stock

Figures A and B, skull fragment with upper dentition, No. 2143 C. I. T. Vert. Pale. Coll. figure A, × 1/3; figure B, slightly smaller than 1/3 natural size. Sespe Uppermost Eocene, California.

Range as representing the youngest Eocene faunal zone in California. Thus, the age determination of the Sespe at locality 292 as uppermost Eocene does not conflict with the evidence furnished by the marine invertebrates in the Coldwater and by the conformable relationship of the latter deposits to the Sespe. Since the Sespe is considerably thicker not far to the east of the section in which the titanothere was found, it is reasonable to regard the age of these continental beds as extending from the uppermost Eocene into at least the Oligocene.

MEASUREMENTS (IN MILLIMETERS) OF No. 2143

Width measured across outer sides of canines			68.8
Length of diastema between C and $P\underline{1}$			19
Length from anterior end of C to posterior end of $M\underline{3}$			283
Medial incisor, width			9.2
Lateral incisor, width			10.4
Canine, width	15.8;	length	19
$P\underline{2}$, transverse diameter	a25.5;	length through middle	a17.6
$P\underline{3}$, transverse diameter	31.7;	length through middle	23.6
$P\underline{4}$, transverse diameter	41.9;	length through middle	29.7
$M\underline{1}$, transverse diameter	45 ;	length through middle	39
$M\underline{2}$, transverse diameter	55 ;	length through middle	52
$M\underline{3}$, transverse diameter	61.7;	length through middle	61.4
Distance from anterior end of snout at point between medial incisors to anterior border of orbit			164
Depth of zygomatic arch immediately in back of postorbital process			51.6

a, approximate.

[1] Stock, C., see these PROCEEDINGS, 1932–1935.

[2] Dreyer, F. E., Thesis entitled: "The Geology of a Portion of Mt. Pinos Quadrangle, Ventura County, California," on file at University of California at Los Angeles.

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AUTOSOMAL LINKAGE IN MAN—THE RECOMBINATION RATIO BETWEEN CONGENITAL TOOTH DEFICIENCY AND HAIR COLOR[1]

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In the course of a search for linked traits, data have been obtained which establish for the first time a clear instance of autosomal linkage[2] in man, provide an estimate of the recombination ratio, and at the same time

elucidate the modes of inheritance of the two traits involved—tooth deficiency and hair color. A brief note on this study was published in 1937 (3). The present paper is based upon further data, and furnishes a fuller statement of material obtained and analysis used.

The method employed for analyzing the data is an adaptation of a technique used earlier by the author. Although the statistical devices proposed by Bernstein in 1931, by Wiener in 1932 and by the author in 1932, have been advanced and refined through the theoretical work of the University of London group (Fisher, Haldane, Hogben, *et al.*), who have provided precise and efficient techniques for detecting linkage and estimating the amount of crossover, the newer methods have required in general:

(1) that the genetic formulae of the parents were either known or could be inferred,

(2) that the modes of transmission of the traits to be studied were well understood,

(3) that the method of ascertainment of cases was known. There are many instances in which none of these conditions hold; when, in fact, the detection of linkage relationships might serve to *clarify* the transmission mechanisms. As this was true of the data to be reported herewith, we reluctantly turned back from the London formulae, and dusted off our earlier study (Burks and Tolman, 1932). Although the results of this investigation had been negative, the method had the advantage of being applicable to complex traits, and of requiring for experimental subjects the members of only a single generation. Briefly, the method involved the comparison of pairs of siblings upon one trait when (*a*) they resembled each other, and (*b*) did not resemble one another upon a second trait or group of traits. If two traits showed no correlation in the general population, but did tend to vary concomitantly within a sibship, evidence for linkage could be adduced.[3]

Eugenics Record Office Study.—Following a clue from a three-generation pedigree published by Beadle in 1926, the indexed repository of family schedules at the Eugenics Record Office was search for cases showing congenital tooth deficiency.[4] Fifteen usable families were found (with at least one affected sibling, and with two or more siblings past the age range in which rapid changes in hair pigmentation are taking place—i.e., past fifteen). Six categories ranging from black to flaxen were employed in these schedules for recording hair color; also three categories of red, which occurred only twice in this material, and was disregarded in the analysis because of its genetic independence of brown or melanic pigment (5).

Members of each sibship were paired in all possible combinations, and the pairs so obtained were arranged fourfoldwise according as the paired offspring were similar or dissimilar in each of the two traits under considera-

tion. Following Mather (6), these separate familial tabulations were combined without weighting. The chi-square technique was applied for testing the significance of deviations from proportionality of the combined fourfold tables. The Boas-Yulean ϕ was also computed as a rough indicator of *closeness* of association.

The basic results for tooth deficiency and hair color are shown in table 1. Subdivision was made of (1) ten families in which a parent and at least one offspring were affected, and (2) the remaining five families. It is reasonable to suppose either that families with and without an affected parent are genetically non-homogeneous with respect to each other, or that the reports

TABLE 1

TOOTH DEFICIENCY TESTED FOR LINKAGE WITH HAIR COLOR: EUGENICS RECORD OFFICE CASES

GROUP	NO. OFFSPRING AFFECTED	NO. OFFSPRING NORMAL	NO. PAIRS	ϕ	CHI-SQUARE*	P	HAIR	TOOTH DEFICIENCY SIMILAR	TOOTH DEFICIENCY DISSIMILAR
15 families, 1 or more affected offspring	28	26	88	0.17	1.75	<0.20	Sim.	14	6
							Dissim.	34	34
10 families, parent and 1 or more affected offspring	22	13	56	0.21	1.47	<0.24	Sim.	8	3
							Dissim.	21	24
5 families, neither parent affected	6	13	32	0.09	0.02	0.90	Sim.	6	3
							Dissim.	13	10

* Yates's correction for continuity has been applied.

are more accurate in the group designating affected parents as well as offspring. For whichever reason, the tests for linkage in the two subgroups give quite different results.

The evidence as a whole appears positive for linkage, i.e., the fourfold tables deviate from proportionality in the expected direction in the case of the total group, and in the subgroup of ten families having an affected parent. In evaluating the probabilities that in fourfold distributions of independent variables, chance deviations would occur as large as those actually found, it must be borne in mind that fluctuations in a direction *favorable* to linkage would occur decidedly less often than the P's indicate—by the order of approximately one-half. The results, while not conclusive, seemed to justify collection of further data.

Field Study.—To enlarge the preliminary study based upon available records, additional families with affected offspring were located through

clinics and interested persons. Care was taken in the selection of propositus cases to secure a group whose missing teeth (incisors or premolars) were almost surely congenitally missing, and not merely extracted, impacted or retarded.

Out of forty-three referred families that could be located and visited, there were fourteen containing two or more siblings over fifteen years of age. When individuals in these families, by inspection, lacked one or more teeth (not accounted for by extraction), confirmation was sought by x-ray. Third molars were at first disregarded entirely, but when chance observations suggested that missing third molars might be genetically related to other missing teeth, and possibly furnish an explanation of apparently "isolated" or non-familial cases of tooth deficiency, they were included thereafter in the diagnostic schedule. In the fourfold tabulations of table 2,

TABLE 2

TOOTH DEFICIENCY TESTED FOR LINKAGE WITH HAIR COLOR: FIELD CASES

GROUP	NO. OFFSPRING AFFECTED	NO. OFFSPRING NORMAL	NO. PAIRS	ϕ	CHI-SQUARE*	P	HAIR	TOOTH DEFICIENCY SIMILAR	TOOTH DEFICIENCY DISSIMILAR
14 families, 1 or more affected offspring	36	16	106	0.22	4.05	<0.05	Sim.	37	8
							Dissim.	38	23
10 families, normals surely differentiated from cases with missing third molars (1 family overlaps with following subgroup)	29** (+2)	5	63	0.25	2.77	<0.10	Sim.	29	3
							Dissim.	22	9
5 families, third molars not diagnosed in otherwise normals (1 overlapping family)	7 (−2)	11	43	0.08	0.026	0.90	Sim.	8	5
							Dissim.	16	14

* Yates's correction for continuity has been applied.

** Of these, 13 cases were affected only in third molars.

affected sibling pairs were counted "similar" regardless of which or how many teeth were congenitally missing.

Hair color was appraised by matching the hair of the back of the head, close to the scalp, against the standard "Shur-on" chart of human hair. We followed the principle of classifying sibling pairs as "similar" in hair color if their shades were so close together on the chart that discrimination between the two shades of hair was doubtful or even liable to a reversal of direction if repeated on another day. All other sibling pairs were counted "dissimilar."[5]

The relation between tooth deficiency and hair color in these families is shown in table 2. The evidence for linkage is somewhat stronger in this array of data than in that based upon the records—in part because a larger number of sibling pairs was available. Again we recall that the prob-

ability that chance deviations from proportionality should be in a direction *favorable* to linkage is of the order of one-half the P value corresponding to chi-square. The two independent sets of data can leave little doubt as to the actual presence of linkage.

Estimate of Recombination Ratio.—In reaching an estimate of the recombination ratio, it is necessary to adopt some reasonable hypothesis as to hereditary mechanisms that will not only account for the sibling distributions of the two traits, but for the *linkage relation* between them. With the two traits under discussion, there is no common agreement as to their modes of transmission. Bearing in mind the different methods by which

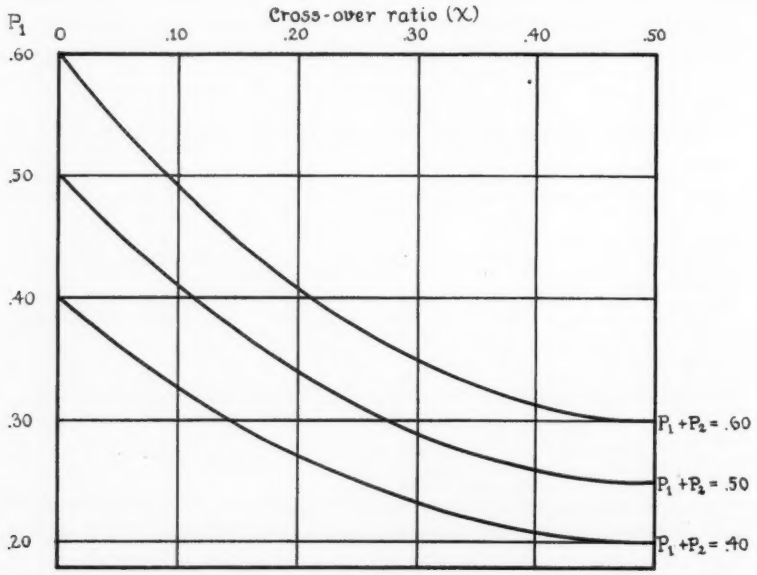

FIGURE 1

Calculated cross-over ratio for given combinations of P_1 and P_2

the E. R. O. cases and the field cases were ascertained and the data collected, the numbers of affected[6] and normal offspring, the proportions of similar and dissimilar pairs on each trait, and the *differences* in similar pairs on trait 1 in comparisons of similar and dissimilar pairs on trait 2 (and vice versa), we may investigate the consequences of the following genetic schema, which fits the data better than any other we were able to devise:

Tooth deficiency: missing third molars due to simplex condition *Aa;* more extensive deficiencies due to duplex condition *AA;* occasional overlap in phenotypes; selective but

irregular suppression of the different teeth comparable to the influence of Dichaete, Hairless, Scute or Echinus in suppressing particular bristles in Drosophila (8).

Hair color: varied and widely distributed series of alleles incompletely dominant with respect to one another, giving (1) in families with two heterozygous parents, four potential phenotypes corresponding to four genotypes [H_1H_3, H_1H_4, H_2H_3, H_2H_4] and (2) in families with one homozygous parent (or families in which certain phenotypes are not readily distinguished), two potential phenotypes corresponding to two genotypes [H_1H_2, H_1H_4].

In table 1, the subgroup for which an affected parent is reported presumably arises from matings $AA \times Aa$. Removing the record of one atypical family sent in by an interested correspondent *because* of the large number of affected members (mother and six of seven offspring), we have the fourfold $\begin{matrix} & +T & - \\ H^{+} & 7 & 2 \\ & -7 & 19 \end{matrix}$ which would seem to conform to hair color plan 1. According to these assumptions, the following equations furnish an estimate of the recombination ratio, χ. For pairs similar in tooth deficiency, proportion similar in hair color,

$$P_1 = \tfrac{1}{2}[\chi^2 + (1 - \chi)^2] \qquad \text{whence } \chi = 0.$$

For pairs dissimilar in tooth deficiency, proportion similar in hair color,

$$P_2 = \tfrac{1}{2}[2\chi(1 - \chi)] \qquad \text{whence } \chi = 0.120.$$

Combining above equations,

$$P_1(2\chi - 2\chi^2) - P_2(2\chi^2 - 2\chi + 1) = 0 \qquad \text{whence } \chi = 0.096.$$

Values of χ corresponding to different combinations of P_1 and P_2 have been calculated from the third equation, and are shown in graphical form in figure 1. It should be noted that diagnosis of third molar cases (i.e., heterozygous cases) is not necessary for estimation of χ in this group if our hypothesis is tenable, since all offspring not AA would then be Aa. Even if our hypotheses of simplex and duplex phenotypes should have to be enlarged to include *some* pedigrees in which the simplex condition is sufficient to produce missing anterior teeth, these pedigrees would contain only two types of offspring (Aa and aa), and the formulae for estimation would remain the same.

The field cases offer additional complications. There is a paucity of information regarding the parent generation, for most of the parents (in the underprivileged groups located through clinics) had lost so many teeth by decay that we could seldom determine whether any were congenitally missing or not. A rough estimate of χ is possible, however. The distributions suggest that the majority of families may be of an $Aa \times Aa$ type of mating, and that the diagnosis of missing third molars in the offspring is

therefore of particular importance. Plan 2 appears to fit the data on hair color better than plan 1, possibly in part because of a different racial composition of families, and in part because our method of classification was likely to merge fine differences.

Using the subgroup in which third molars were diagnosed, we may calculate the numbers of sibling pairs who are presumably *AA–AA*, *AA–Aa*, *Aa–Aa*, according to our hypothesis, and from these, the proportion similar in hair color to be expected among all affected pairs for different values of χ. The proportion, 0.588, in the present material would correspond to χ between 0.10 and 0.15.

The same procedure might equally well be followed with the sibling pairs having one affected and one normal member. The present data are hardly numerous enough to justify this calculation, though it is interesting to note that the data as they stand correspond to an estimated recombination ratio between zero and 0.05. The fluctuations of these various estimates are no greater than sampling errors could account for, and indeed the general *convergence* of estimated values of χ from the different data of differing genetic composition lends strong support to the genetic formulation advanced.

Further investigation may possibly require modification of our genetic interpretation, with a shift in estimated χ. The present data considered, it seems probable that the actual recombination ratio lies not far from 0.10.

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[1] Grateful acknowledgment is made for counsel and facilities provided by Drs. C. B. Davenport and H. H. Laughlin of the Eugenics Record Office. Dr. John O. McCall, director of the Murry and Leonie Guggenheim Dental Clinic opened the files of the clinic to our field workers, and generously provided dental x-rays of patients and members of their families whom we referred. Through Dr. William Hemley, director of the Orthodontia Clinic of New York University Hospital, several additional families were secured for investigation. Members of the staffs of both clinics showed us every courtesy and consideration, as did the dentists in private practice to whom we referred cases for x-ray. The writer also expresses appreciation for the contributions of Mrs. Jean C. Challman and Miss Natalie M. Raymond who assisted in gathering the field data.

[2] The possibilities of sex linkage or incomplete sex linkage, or of correlation between tooth deficiency and hair color were excluded at the outset by appropriate tests.

[3] Penrose (7) has independently proposed a similar scheme. The present data have been tabulated in the "fourfold table" form employed by Penrose, but the treatment of data has varied from his, and from our earlier treatment, in a number of respects.

[4] The teeth most frequently reported as missing were the permanent upper lateral incisors, though lower incisors and upper or lower pre-molars were often missing either singly or in various combinations. Affected offspring in individual families often varied among themselves in the location and degree of involvement. Only one individual was reported as having missing third molars (this being in conjunction with other tooth deficiencies). We have reason to believe that missing third molars often pass unnoticed.

[5] To guard against any possible bias, family records of hair color, with names and notations on teeth detached, were submitted to Jean C. Challman, whose classification, corroborated by the writer, was used in the final tabulation.

[6] The data of the first subgroup in table 2 were further subdivided to demonstrate the common genetic derivation of missing third molars and other missing teeth. The number of cases is not large enough to establish the proportions with great accuracy, but pairs in which both members are affected, regardless of which teeth, appear to be definitely more similar in hair color than pairs having one affected and one normal member. For this table chi-square = 8.8014, $N = 4$, P is between 0.10 and 0.05.

	BOTH LACKING TEETH OTHER THAN THIRD MOLARS	BOTH LACKING THIRD MOLARS	1 LACKING "OTHER" 1 LACKING MOLARS	1 LACKING "OTHER" 1 NORMAL	1 LACKING MOLARS 1 NORMAL
Number of pairs	15	17	19	7	5
Proportion similar in hair color	0.40	0.76	0.58	0.29	0.20

REMARKS ON PLATEAU'S AND DOUGLAS' PROBLEM

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The objective of Plateau's Problem in the generalized form by J. Douglas[1] is the proof of the existence of a minimal surface of given topological structure and with k prescribed boundary curves. Its solution has been the subject of several recent publications.[2] Therefore it may be justified to give the following account of a different and simplified version of the existence proof contained in a forthcoming paper on this and related subjects.

Minimal surfaces in the n-dimensional space with the coördinates $x_1, \ldots, x_n$ or the position vector $\mathfrak{x}$ are represented by functions x_ν of two parameters u, v in a domain B over the plane of the complex variable $w = u + iv$, in such a way that the x_ν are harmonic in B:

$$\Delta \mathfrak{x} = \mathfrak{x}_{uu} + \mathfrak{x}_{vv} = 0$$

and that $\mathfrak{x}_u^2 - \mathfrak{x}_v^2 = \mathfrak{x}_u \mathfrak{x}_v = 0$. Since for harmonic vectors $\mathfrak{x}$ the expression $\varphi(w) = \mathfrak{x}_u^2 - \mathfrak{x}_v^2 - 2i\mathfrak{x}_u\mathfrak{x}_v$ is analytic in w this second condition is simply represented by

$$\varphi(w) = \Sigma f_\nu'(w)^2 = 0.$$

Here $f_\nu(w)$ denotes the analytic function whose real part is x_ν. We now consider domains B of the prescribed topological structure. The boundary C of B consists of k curves $C_1, \ldots, C_k$ which will be made to correspond monotonically to the k prescribed curves $\Gamma_1, \ldots, \Gamma_k$ in the x-space.

The solution of the Plateau Problem is obtained by solving the following variational problem: Let $\mathfrak{x}(u, v)$ be a vector continuous in $B + C$ which maps C_ν in monotonically on Γ_ν, has piecewise continuous first derivatives in B and for which the Dirichlet Integral

$$D[\mathfrak{x}] = \int\int (\mathfrak{x}_u^2 + \mathfrak{x}_v^2) du dv$$

exists.

It is required to find B and $\mathfrak{x}$ so that $D[\mathfrak{x}]$ attains its lower limit d. In principle B could range over the class of all Riemann domains of the prescribed topological structure. But to avoid use of theorems on conformal mapping one restricts B to a class with smooth boundary curves, but still suitably general. In previous publications I have chosen plane domains B, e.g., for genus zero, domains bounded by k circles, or planes with k finite parallel slits; for higher topological structure slit domains were used, or fundamental domains of a Schottky group from which k circular discs are removed.[3]

In this paper, instead, we consider Riemann domains B with more sheets over the w-plane so that each boundary curve C_ν is the unit circle. In particular, for the genus zero B may consist of k unit circles connected by means of branch points of total multiplicity $2k - 2$, e.g., $2k - 2$ simple branch points, so that each circular disc is connected with its "next" by a "branch line."[4] If the genus is p we affix to such a domain p different full planes, each by means of 4 simple branch points. But, of course, all limiting cases, hence branch points of higher order, must be admitted. For the case of non-orientability one can easily construct similar normal domains by considering the corresponding orientable double surface.

The solution of the Plateau Problem consists of two steps. First we have to show that the variational problem has a solution—which because of the Dirichlet Principle must necessarily be a harmonic vector $\mathfrak{x}$. Secondly the relation $\varphi(w) = 0$, i.e., the character of the solution as a minimal surface must be proved.

The first step can be achieved and at the same time the lower semi-continuity of the lower bound d in its dependence on Γ_ν be proved under the condition: no minimizing sequence of vectors $\mathfrak{x}_m$ for which $D[\mathfrak{x}_m]$ tends to the lower limit d can tend to degeneration. Tendency to degeneration means that there exists on the surface $\mathfrak{x}_m$ a closed curve L_m which, on $\mathfrak{x}_m$, cannot be continuously deformed into a point and whose diameter tends to zero with m tending to infinity. This condition insures for a minimizing sequence of domains B_m and vectors $\mathfrak{x}_m$ the existence of a limiting domain B of the same type and at the same time the equi-continuity of the boundary values of $\mathfrak{x}_m$; the proof then proceeds without difficulty. The above condition is shown to be equivalent to sufficient conditions in form of inequalities used by Douglas and the author. This is done by means of the following lemma: If there exists a minimizing sequence tending to degeneration there also exists such a sequence all of whose members are actually degenerated; the curves L_m then are replaced by single points.

The advantage of our choice for B appears in the second step, the proof of $\varphi(w) = 0$ for the solution $\mathfrak{x}$ of the variational problem. First, by variation of the boundary values we obtain as in the previous paper the result that $w^2\varphi(w)$ is regular and real along all the boundary circles C_ν of B. Furthermore we observe that $\varphi(w) = \Sigma f_\nu'(w)^2$ has a pole of order not higher than $2r$ in a branch point of order r. For, if the neighborhood of such a branch point, e.g., $w = 0$ is mapped by $w = \rho^{r+1}$ to the simple neighborhood of $\rho = 0$ in the ρ-plane we have

$$\varphi(w) = \left(\frac{1}{r+1}\right)^2 \frac{1}{\rho^{2r}} \sum \left(\frac{df_\nu}{d\rho}\right)^2$$

and $\sum \left(\frac{df_\nu}{d\rho}\right)^2$ is regular around the point $\rho = 0$.

The variability of B consists in the variability of the branch points, and the corresponding variational condition for the solution $\mathfrak{x}$, found by a simple variation, is: $\varphi(w)$ has in a branch point of order r a pole of order not higher than r instead of the *a priori* possible order $2r$.

Now $\varphi(w) = 0$ is proved as follows: Suppose first B to be of genus zero and assume that $\varphi(w)$ does not vanish identically; then $\varphi(w)$ has in $B + C$ a finite number of zeros. N may be their—non-negative—number in B and L their number on C. The total number P of poles in B is, because of our variational condition, not greater than $2k - 2$, the total multiplicity of all branch points. The difference $N - P$ is $1/2\pi$ times the total variation of the arcus of $\varphi(w)$ if w describes the k boundary unit circles in the positive sense circumventing the L zeros of $\varphi(w)$ on C by small semicircles in B. Each of those semicircles contributes the negative amount

$-1/2$. The variation of the arcus of $w^2\varphi(w)$ along the other parts of the circles is zero because this expression is real. Subtracting the variation for w^2, that is, $2k$ times 2π, we obtain

$$N - P = -2k - 1/2L$$

and since $P \leq 2k - 2$ we have $N \leq -2$, which is a contradiction to $N \geq 0$. Hence $\varphi(w) = 0$ is proved.

In the case of genus p the same reasoning holds. We have here $P \leq 2k - 2 + 4p$ because the right-hand side is the total multiplicity of branch points in B. Now $\varphi(w)$ is regular at the point at infinity in each of the p attached planes and has there a zero of at least 4th order, as easily seen from the definition of $\varphi(w)$. Hence *a priori* we have $N \geq 4p$, while as above $N - P \leq -2k$. This leads to the contradiction $N \leq 4p - 2$.

A similar reasoning holds for non-orientable surfaces.

In the case of genus zero the following mapping theorem results as a byproduct for $n = 2$: Every plane k-fold connected domain can be mapped conformally to a k-fold unit circle B. To establish this theorem our general theory has to be supplemented by a verification of the sufficient condition, which can be done by a reasoning similar to that on page 707 f. in the paper quoted in footnote 1.

While also for higher topological structure—not only for genus zero—our present proof proceeds without use of conformal mapping it should be pointed out that by means of mapping theorems in these higher cases a more satisfactory result is obtained—and this, as is shown in the paper quoted above, even in a simpler way. It seems as if the difficulty arising from the artificial reference to particular classes of domains of representation B cannot be eliminated otherwise. However, this difficulty does not occur if degeneration is *a priori* excluded by stronger inequality conditions, as M. Shiffman has done in his analysis of the possibility of relative minima.[5]

[1] *Bull. Am. Math. Soc.*, **36**, 50 (1930).

[2] 1. Douglas, *Jour. Math. and Phys.*, **15**, p. 55 ff., Feb. (1936). 2. Douglas, *Ibid.*, 105 ff., (June, 1936). 3. R. Courant, *Proc. Nat. Acad. Sci.*, **22**, 367 (June, 1936). 4. R. Courant, *Ann. Math.*, **38**, 679 (1937). 5. Douglas, *Proc. Nat. Acad. Sci.*, **24**, 343 (1938).

No. 1 gives results. No. 2 goes on to give details and proofs. No. 5 gives a summary of a detailed exposition in a forthcoming comprehensive paper. No. 3 gives two different general methods, and details for the simplest case. No. 4 contains the complete discussion of the problem for genus zero and sufficient detail for higher genus.

Douglas' method—developed since 1929—starts with a variational problem of the Dirichlet type but for harmonic functions only; this restriction permits consideration of the functional in its dependence on boundary values and thus avoids certain difficulties of the calculus of variations in two dimensions. The methods in Nos. 3, 4 and the present note are essentially based on a two-dimensional variational problem. As

shown in *Proc. Nat. Acad. Sci.*, **24**, 97 (1938), they also serve to solve the problem with free boundaries. Also a forthcoming paper of M. Shiffman should be mentioned. (See footnote 5.)

[3] It is preferable not to assume—as in the paper No. 4 quoted above—that these fundamental domains be bounded by circles, but rather to leave these boundaries free.

[4] It is even permitted to assume all these branch lines parallel.

[5] *Ann. Math.* (1938) and the forthcoming paper quoted in footnote 2. In this paper (doctoral thesis) the domain B is chosen as a slit domain as in the paper No. 4, but with the essential difference that relative minima are treated, and that the variation for the slit domains is carried out without use of conformal mapping.

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